SEQ ID NO:1:
SEQ ID NO:2:

```
ATG AGT AAA GGA GAA GAA CTT TTC ACT GGA GTT GTC CCA ATT CTT GTT     48
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
  1               5                  10
GAA TTA GAT GGT GAT GTT AAT GGG CAC AAA TTT TCT GTC AGT GGA GAG     96
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
            20                  25                  30
GGT GAA GGT GAT GCA ACA TAC GGA AAA CTT ACC CTT AAA TTT ATT TGC    144
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
        35                  40                  45
ACT ACT GGA AAA CTA CCT GTT CCA TGG CCA ACA CTT GTC ACT ACT TTC    192
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
    50                  55                  60
TCT TAT GGT GTT CAA TGC TTT TCA AGA TAC CCA GAT CAT ATG AAA CGG    240
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65                  70                  75                  80
CAT GAC TTT TTC AAG AGT GCC ATG CCC GAA GGT TAT GTA CAG GAA AGA    288
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
                85                  90                  95
ACT ATA TTT TTC AAA GAT GAC GGG AAC TAC AAG ACA CGT GCT GAA GTC    336
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
            100                 105                 110
AAG TTT GAA GGT GAT ACC CTT GTT AAT AGA ATC GAG TTA AAA GGT ATT    384
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
        115                 120                 125
```

FIG. 1A

```
GAT TTT AAA GAA GAT GGA AAC ATT CTT GGA CAC AAA TTG GAA TAC AAC      432
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
    130                 135                 140

TAT AAC TCA CAC AAT GTA TAC ATC ATG GCA GAC AAA CAA AAG AAT GGA      480
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145                 150                 155                 160

ATC AAA GTT AAC TTC AAA ATT AGA CAC AAC ATT GAA GAT GGA AGC GTT      528
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
                165                 170                 175

CAA CTA GCA GAC CAT TAT CAA CAA AAT ACT CCA ATT GGC GAT GGC CCT      576
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
            180                 185                 190

GTC CTT TTA CCA GAC AAC CAT TAC CTG TCC ACA CAA TCT GCC CTT TCG      526
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
        195                 200                 205

AAA GAT CCC AAC GAA AAG AGA GAC CAC ATG GTC CTT CTT GAG TTT GTA      672
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
    210                 215                 220

ACA GCT GCT GGG ATT ACA CAT GGC ATG GAT GAA CTA TAC AAA TA           716
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
```

FIG. 1B

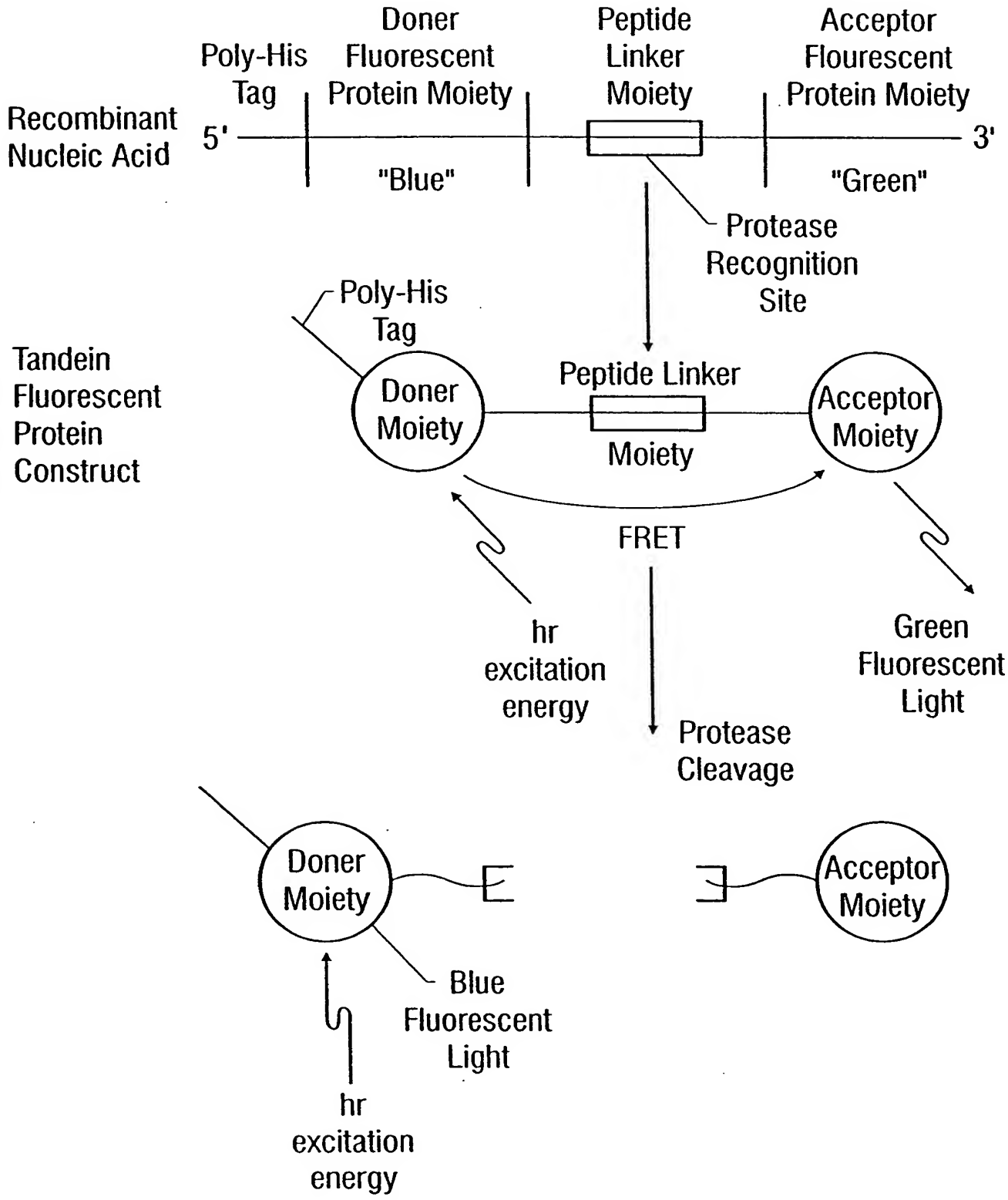
Recombinant Nucleic Acid
5'
Poly-His Tag
Doner Fluorescent Protein Moiety
"Blue"
Peptide Linker Moiety
Acceptor Flourescent Protein Moiety
"Green"
3'
Protease Recognition Site
Tandein Fluorescent Protein Construct
Poly-His Tag
Doner Moiety
Peptide Linker Moiety
Acceptor Moiety
FRET
hr excitation energy
Green Fluorescent Light
Protease Cleavage
Doner Moiety
Acceptor Moiety
Blue Fluorescent Light
hr excitation energy

FIG. 2

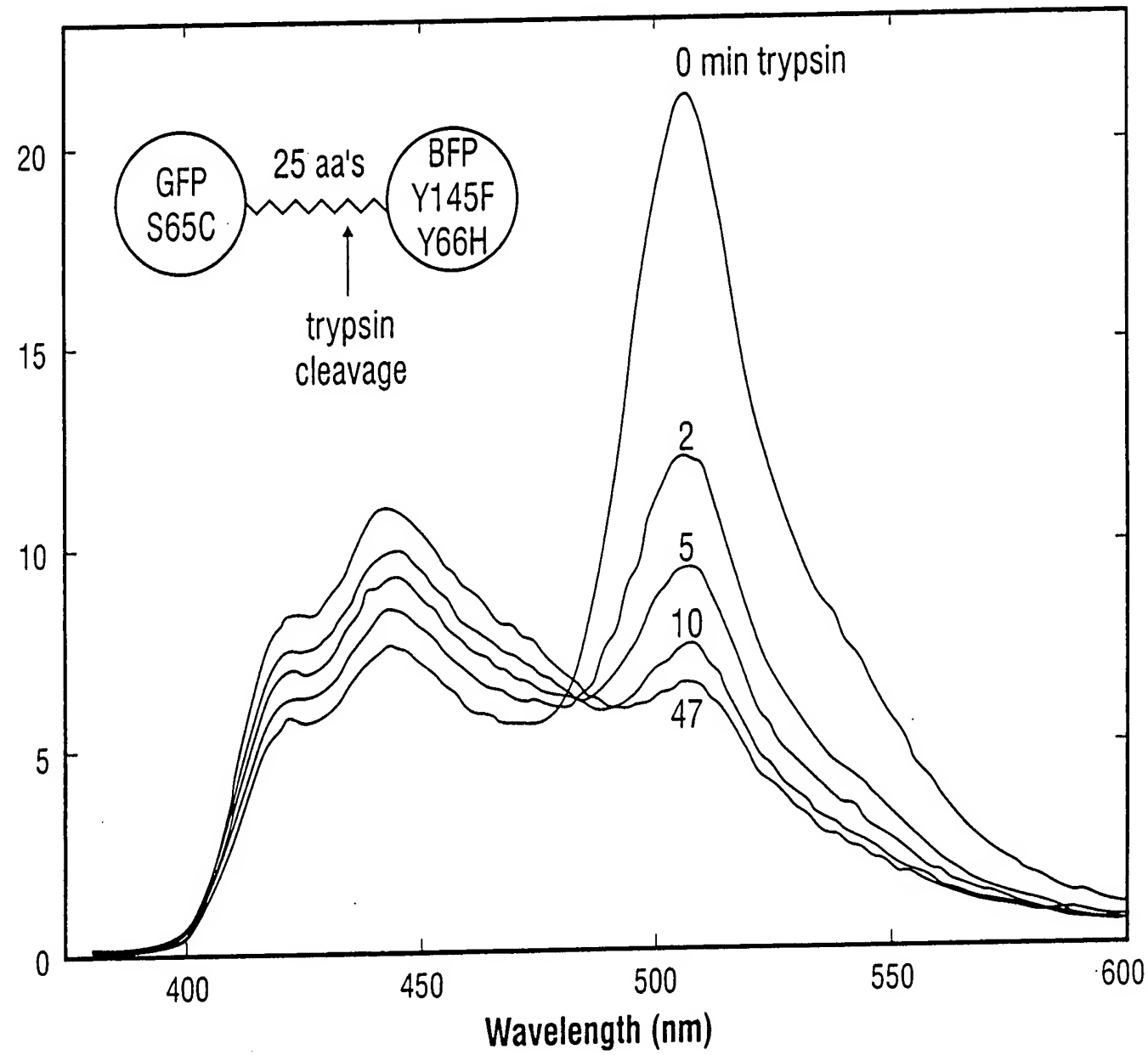
0 min trypsin
GFP
S65C
25 aa's
BFP
Y145F
Y66H
trypsin
cleavage
2
5
10
47
20
15
10
5
0
400
450
500
550
600
Wavelength (nm)
Fluorescence
Intensity

FIG. 3

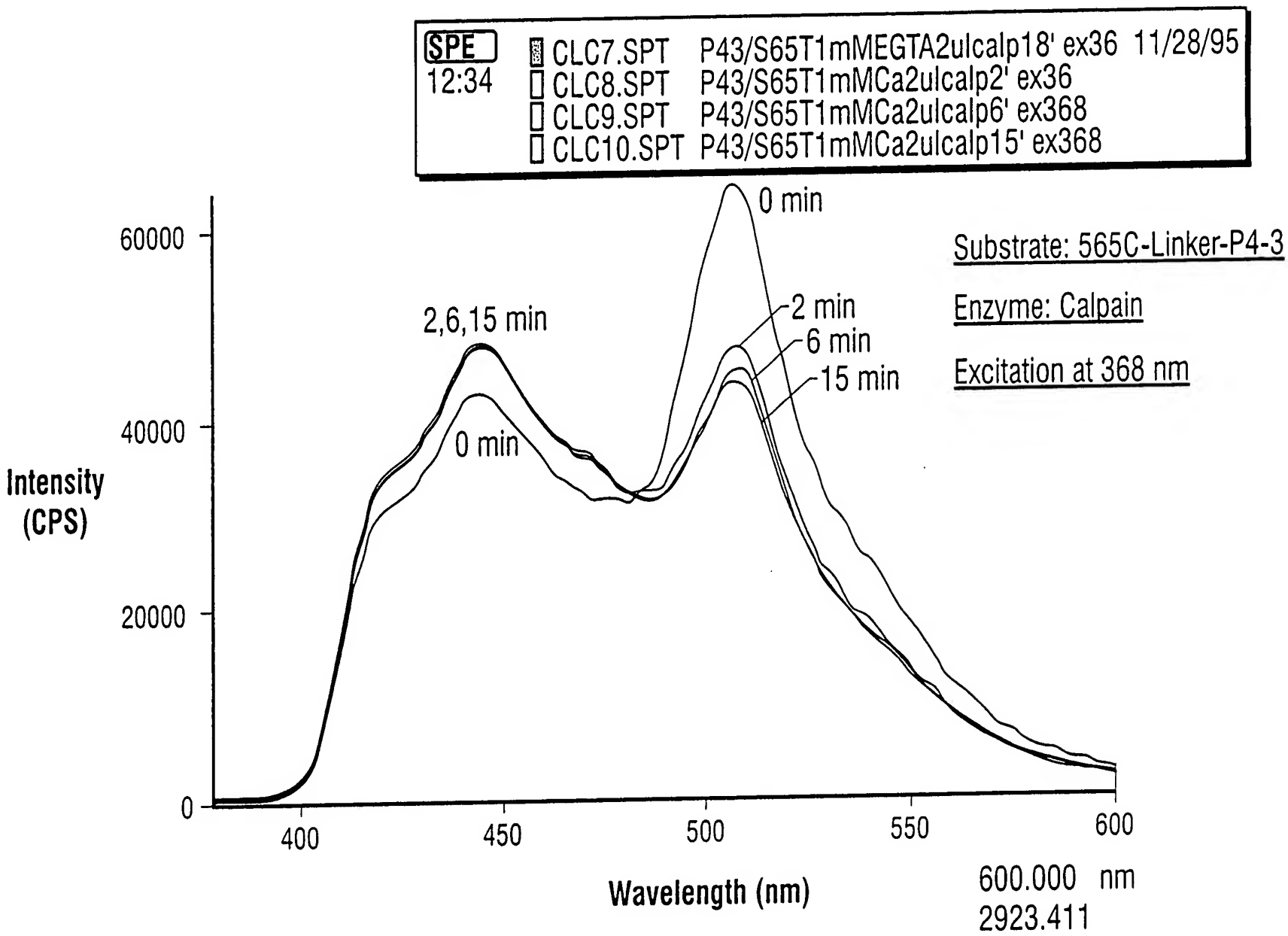
SPE
12:34
CLC7.SPT P43/S65T1mMEGTA2ulcalp18' ex36 11/28/95
CLC8.SPT P43/S65T1mMCa2ulcalp2' ex36
CLC9.SPT P43/S65T1mMCa2ulcalp6' ex368
CLC10.SPT P43/S65T1mMCa2ulcalp15' ex368
0 min
2,6,15 min
0 min
2 min
6 min
15 min
Substrate: 565C-Linker-P4-3
Enzyme: Calpain
Excitation at 368 nm
60000
40000
20000
0
Intensity (CPS)
400
450
500
550
600
Wavelength (nm)
600.000 nm
2923.411

FIG. 4

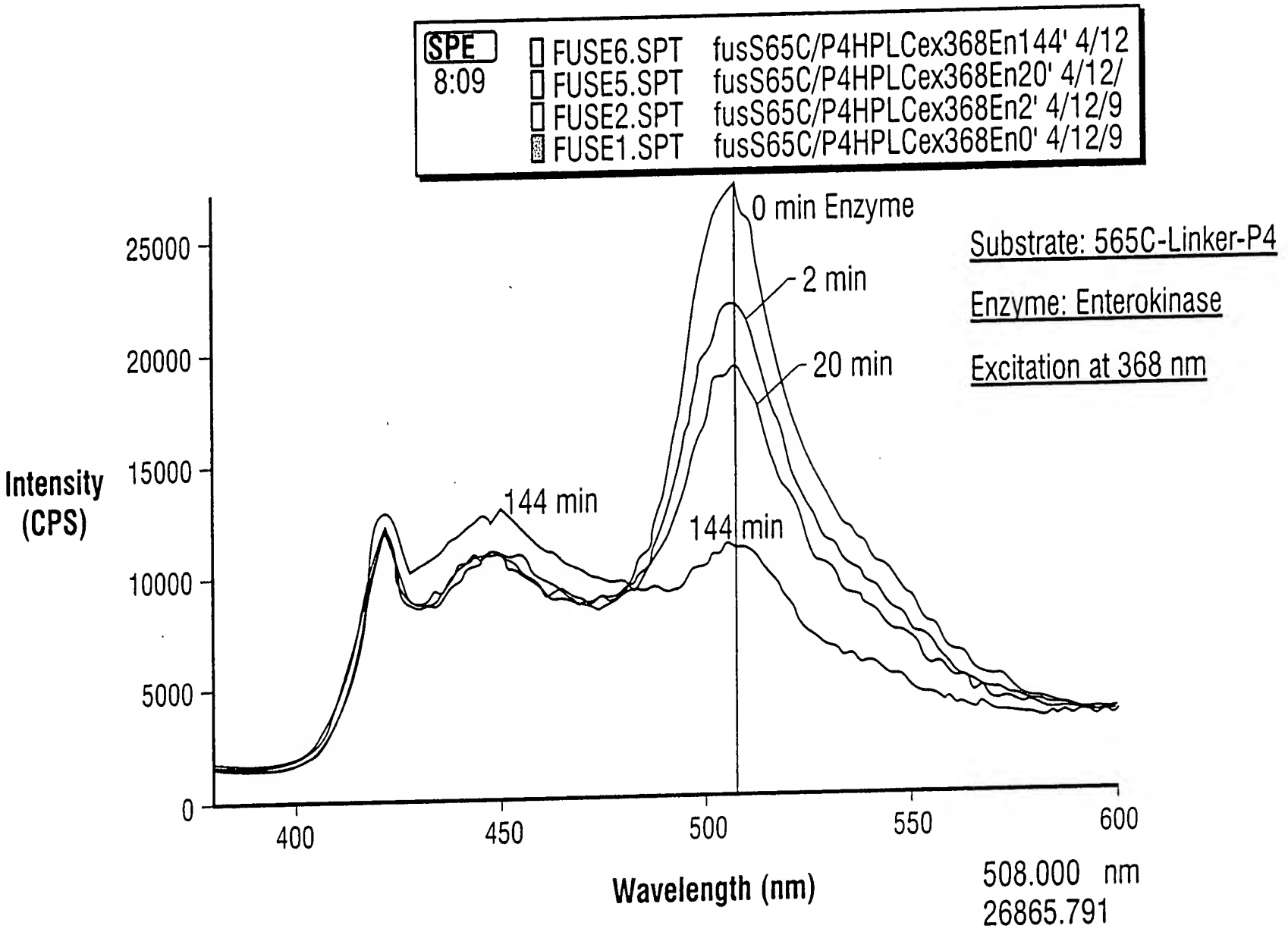
SPE
8:09
FUSE6.SPT fusS65C/P4HPLCex368En144' 4/12
FUSE5.SPT fusS65C/P4HPLCex368En20' 4/12/
FUSE2.SPT fusS65C/P4HPLCex368En2' 4/12/9
FUSE1.SPT fusS65C/P4HPLCex368En0' 4/12/9
0 min Enzyme
2 min
20 min
144 min
144 min
Substrate: 565C-Linker-P4
Enzyme: Enterokinase
Excitation at 368 nm
Intensity (CPS)
25000
20000
15000
10000
5000
0
400
450
500
550
600
Wavelength (nm)
508.000 nm
26865.791

FIG. 5

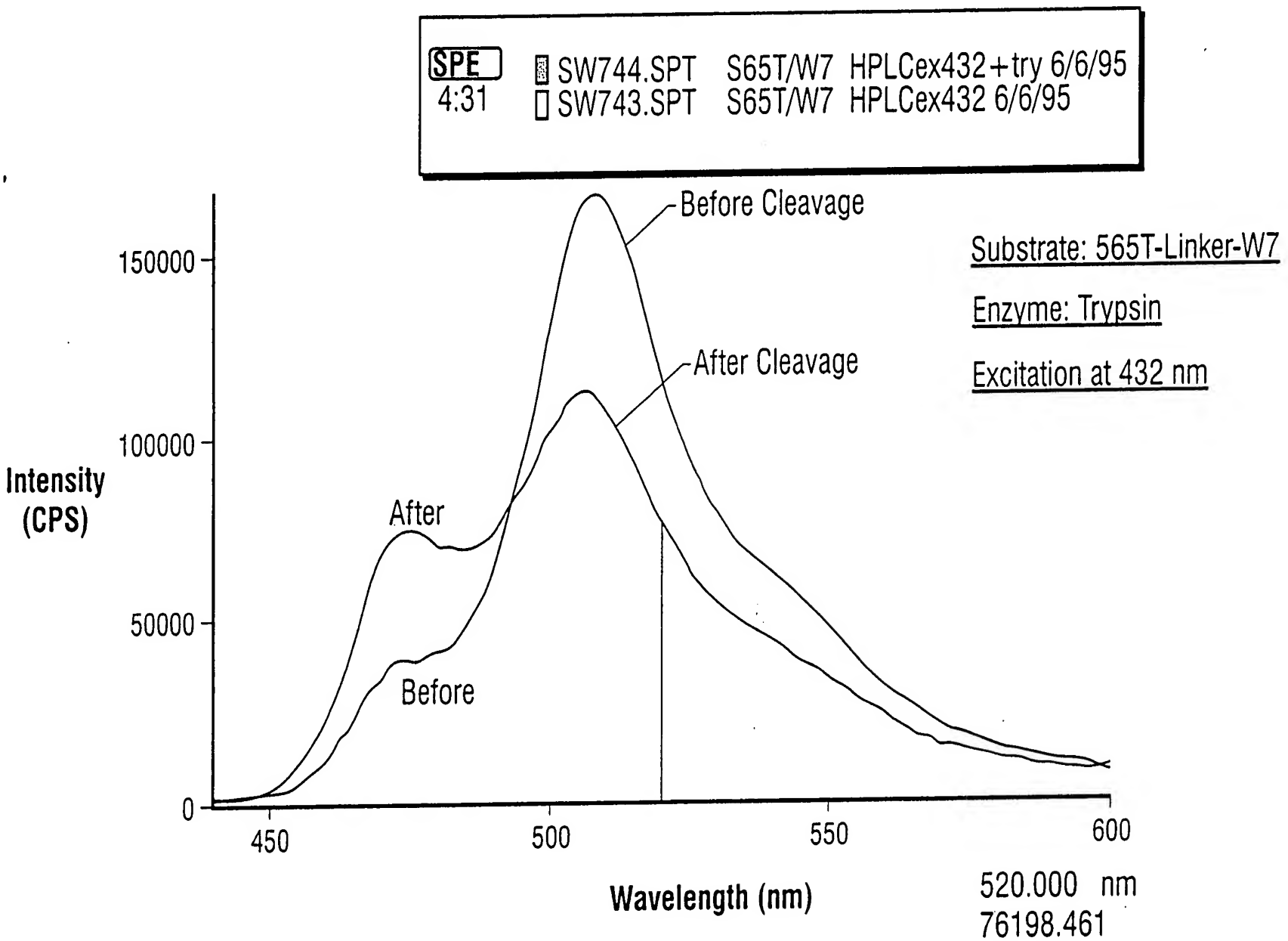
SPE
4:31
SW744.SPT S65T/W7 HPLCex432+try 6/6/95
SW743.SPT S65T/W7 HPLCex432 6/6/95
Before Cleavage
After Cleavage
Substrate: 565T-Linker-W7
Enzyme: Trypsin
Excitation at 432 nm
150000
100000
50000
0
Intensity (CPS)
After
Before
450
500
550
600
Wavelength (nm)
520.000 nm
76198.461

FIG. 6

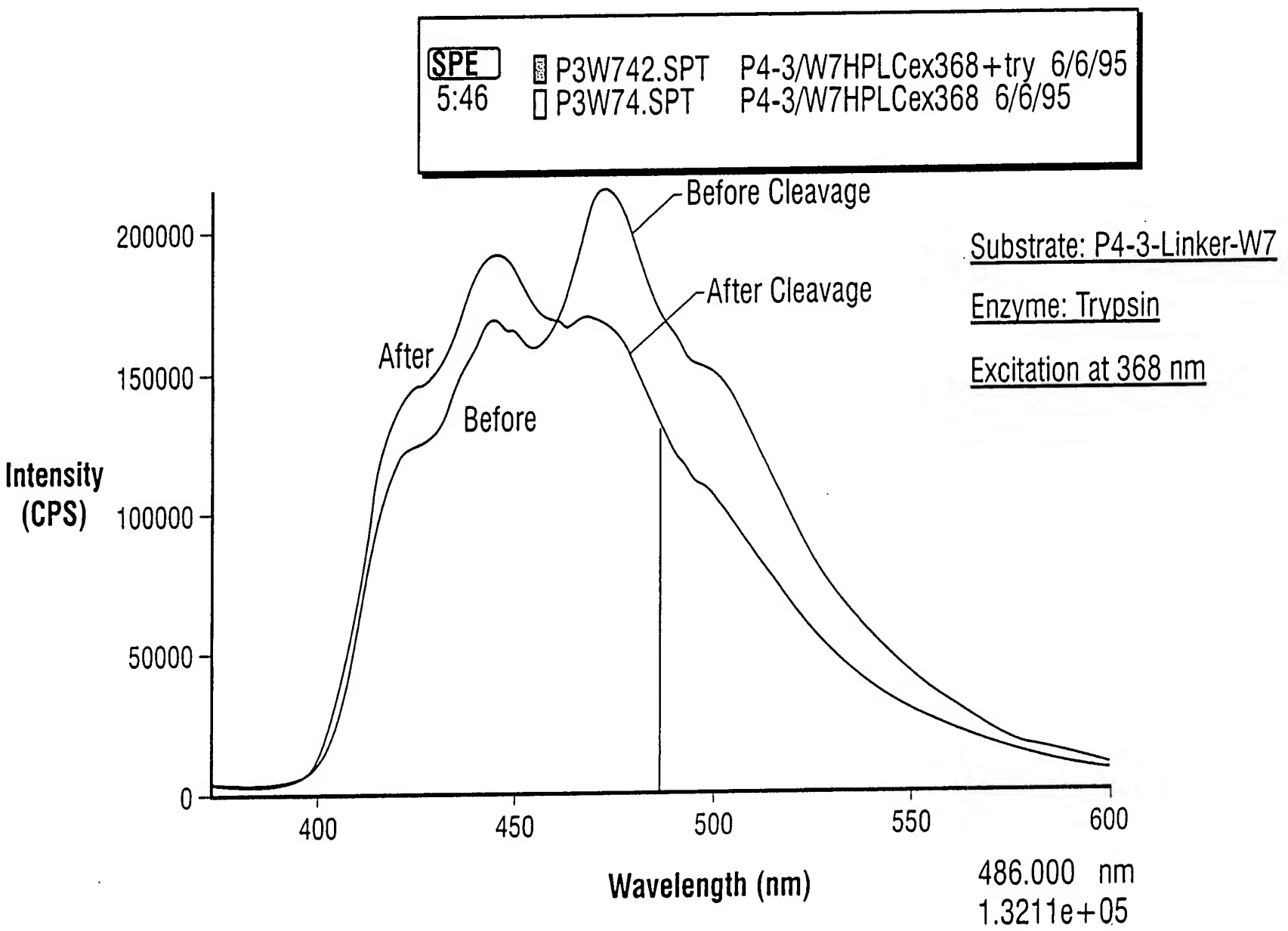
SPE
5:46
P3W742.SPT P4-3/W7HPLCex368+try 6/6/95
P3W74.SPT P4-3/W7HPLCex368 6/6/95
Before Cleavage
After Cleavage
After
Before
Substrate: P4-3-Linker-W7
Enzyme: Trypsin
Excitation at 368 nm
Intensity (CPS)
200000
150000
100000
50000
0
400
450
500
550
600
Wavelength (nm)
486.000 nm
1.3211e+05

FIG. 7

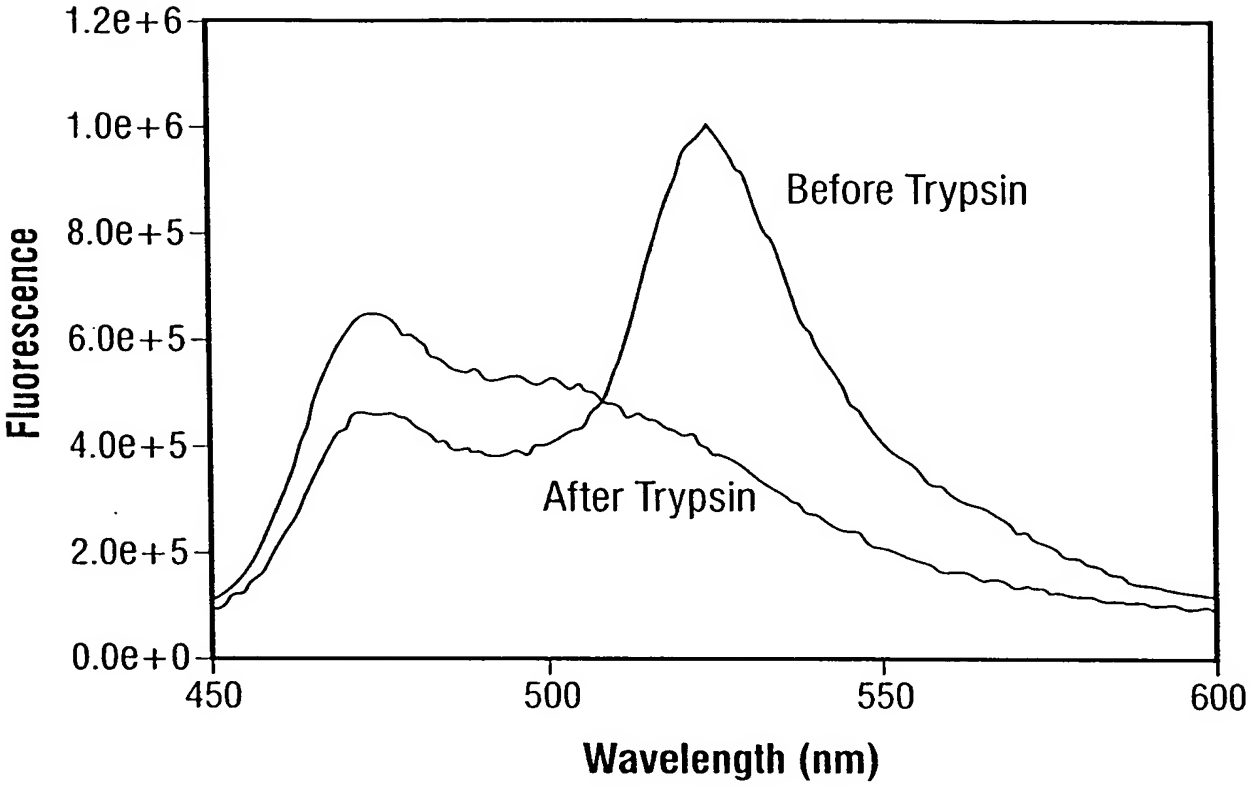

FIG. 8

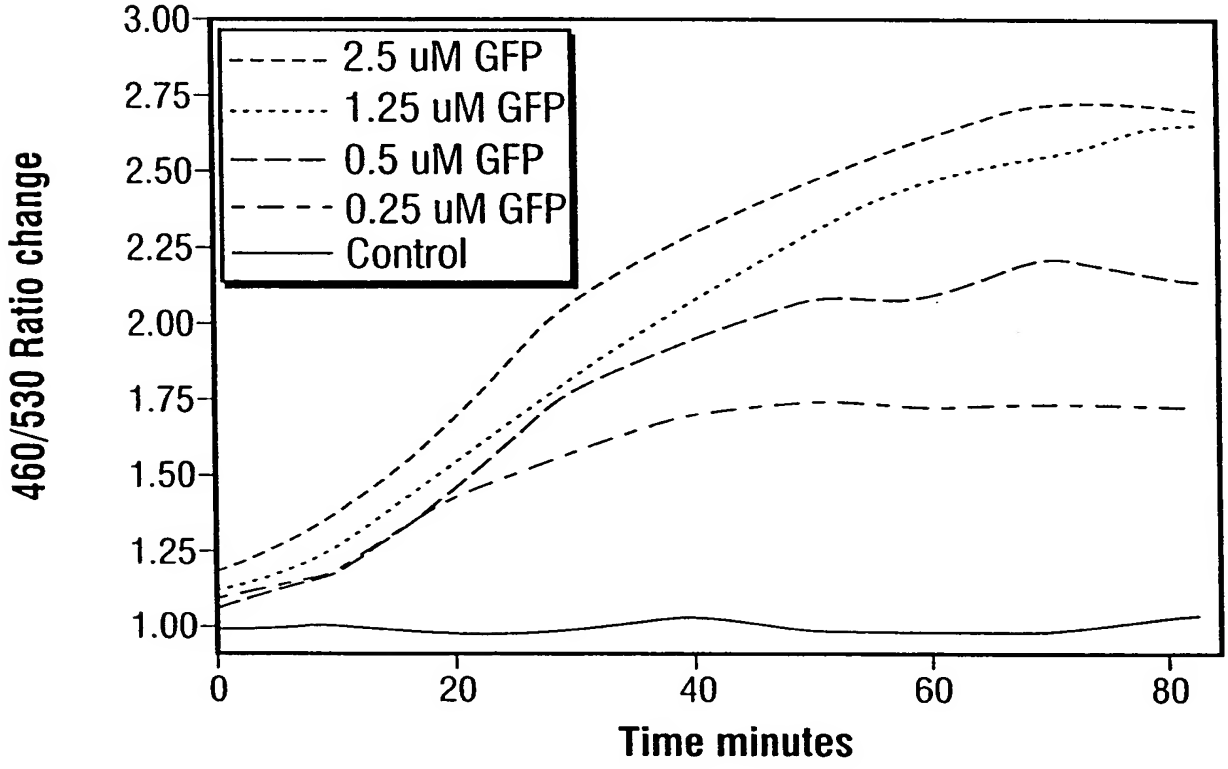

FIG. 9

20020218.0505/5005.0125.02

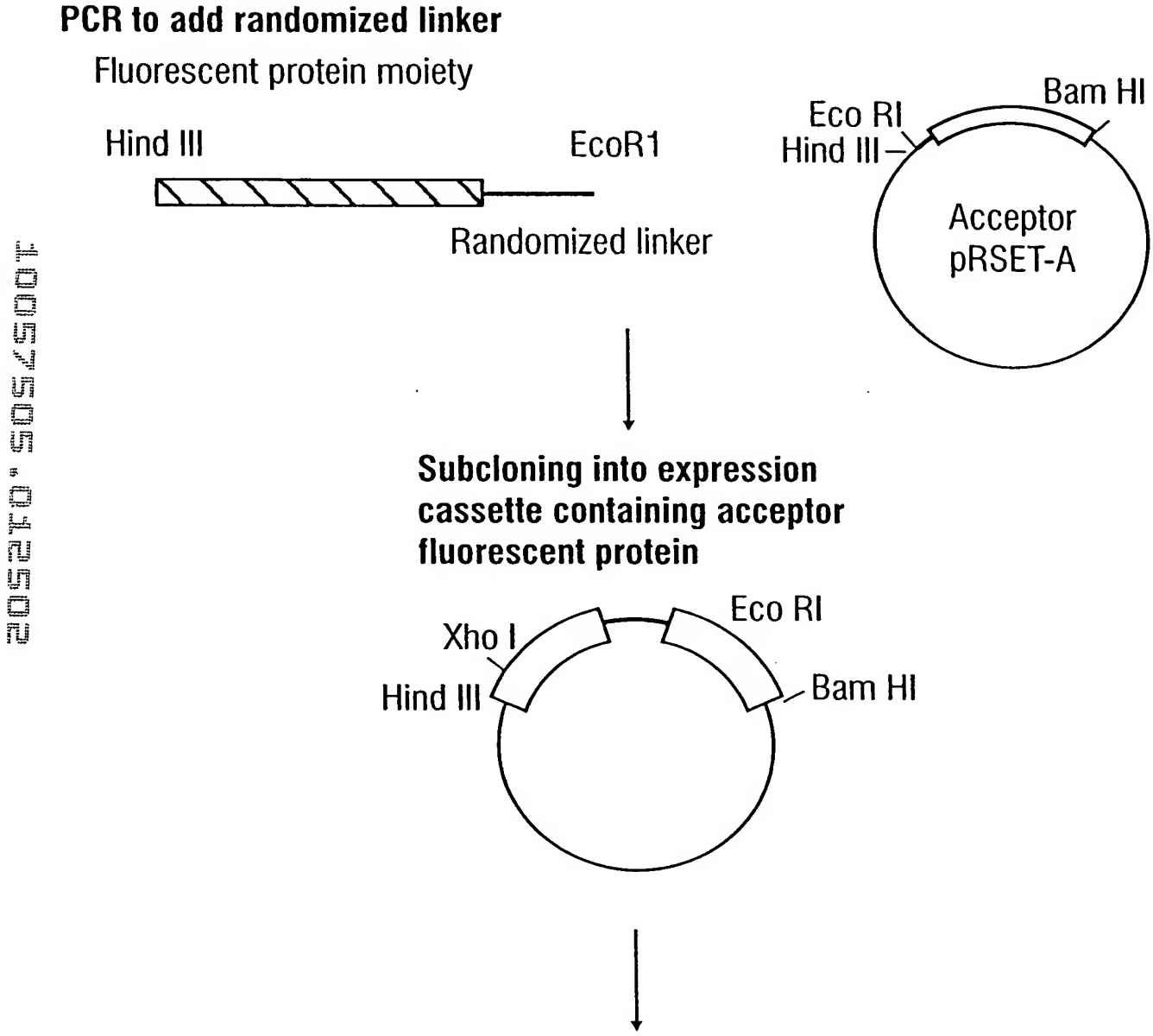
PCR to add randomized linker
Fluorescent protein moiety
Hind III
EcoR1
Randomized linker
Eco RI
Bam HI
Hind III
Acceptor
pRSET-A
Subcloning into expression cassette containing acceptor fluorescent protein
Xho I
Eco RI
Hind III
Bam HI
Transfection and Screening in cells to identify sequences that are cleaved when co-expressed with the target enzyme

FIG. 10